Lysozymuria and acute disorders of renal function

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SYNOPSIS On the assumption that increased urinary lysozyme concentration ('lysozymuria') indicates tubular proteinuria and therefore impaired tubular function, urinary lysozyme has been estimated in acute disorders where transient disturbances of renal function might be expected, in cases diagnosed clinically as extrarenal uraemia, and in a few examples of acute renal disease. Reversible lysozymuria occurred with hypokalaemia, postoperative 'collapse', electrolyte depletion, severe extrarenal infection, acute pyelonephritis, the nephrotic syndrome, after a few apparently uncomplicated surgical operations, and very transiently after ventricular fibrillation abolished by DC shock. There was no lysozymuria with severe uraemic heart failure, aspirin and paracetamol poisoning, or severe jaundice, nor in two cases of acute glomerulonephritis. Although lysozymuria may occasionally be useful in the clinical diagnosis of acutely disordered renal function, the results suggest that its value is limited; on the other hand, they have provided information on renal pathophysiology in acute disease.

There is good evidence that the excretion of low molecular weight proteins is increased when renal tubular function is impaired (Flynn and Platt, 1968); the result is 'tubular proteinuria'. Different low molecular weight proteins seem to follow the same pattern of urinary excretion in health and disease (Harrison, Lunt, Scott, and Blainey, 1968). These proteins all pass more or less readily into the glomerular filtrate and are believed then to be re-absorbed, mainly if not entirely in the proximal tubules, so that they scarcely appear in normal urine. Decreased tubular reabsorption will therefore cause an increase in their urinary concentration, and when this occurs it is probably due to generalized tubular cell damage since specific defects of tubular protein transport have not so far been described.

Lysozyme (molecular weight 15 000) is such a protein; its assay happens to be simple, sensitive, and subject to few complicating factors, and in some circumstances urinary lysozyme concentration can be one of the most useful indices of tubular function (Adams, Harrison, and Scott, 1969). Lysozyme in the blood probably originates in the leucocytes (Jollès, Sternberg, and Mathé, 1965) and is probably concerned in defence against bacterial infection, but in studies of renal function these considerations are unimportant because the enzyme may be regarded simply as an example of a low molecular weight

protein. There is so far no evidence that lysozyme is secreted into the urinary tract; pyuria might be expected to increase the urinary lysozyme concentration, but any effect there may be has so far proved to be very slight (Barratt and Crawford, 1970). Nevertheless to establish that lysozymuria is due to impaired tubular reabsorption it is necessary to know that the normal renal threshold is not exceeded, because when this occurs, as in some forms of leukaemia, there is 'overflow' lysozymuria analogous to the glycosuria of diabetes (Osserman and Lawlor, 1966).

In renal disease, tubular proteinuria and lysozymuria were first described in relation to chronic disorders of function (Prockop and Davidson, 1964); both are always found in advanced chronic renal failure and are useful in the diagnosis of cadmium nephropathy. Acute, reversible tubular proteinuria and lysozymuria have been reported in acute tubular necrosis (Révillard and Manuel, 1965; Noble and Brainerd, 1966; Maiorca and Scarpioni, 1968; Hayslett, Perillie, and Finch, 1968; Wauters and Favre, 1970), as a sign of renal transplant rejection (Shehadeh, Carpenter, Monterio, and Merrill, 1970; Hansen and Weeke, 1970; Harrison, Barnes, and Blainey, 1972), and after exercise (Poortmans, 1972). The present study was undertaken to find out whether lysozymuria is common among non-oliguric patients in ordinary hospital wards, by studying three main groups of cases: (a) patients with con-

ditions which when severe or complicated might be expected to cause tubular damage; (b) patients with a clinical diagnosis of extrarenal uraemia; and (c) a few patients with acute renal disease.

Method

The assay was a modification of the method of Smolelis and Hartsell (1949). The substrate was a suspension of *Micrococcus lysodeikticus* cells (Sigma Chemical Co), 25 mg/ml. Sodium phosphate buffer, pH 6.2, 0.067 M, was used for diluting the substrate and standards. Samples of 0.5 ml were added to 5.0 ml substrate suspension; optical densities at 450 nm were measured at 0.5 min and 10 min, and the reaction was allowed to proceed at 37°. The difference between the OD readings was referred to a standard curve constructed from samples containing egg-white lysozyme in buffer (Seravac Laboratories) covering the range 1.0–20.0 µg/ml. Urines were diluted if necessary; to avoid the inhibitory effect of macromolecules in serum (Harrison and Swingler, 1971) all sera were diluted 1 in 8 routinely before estimation. For lysozyme in urine the coefficient of variation was 7%. Lysozyme activity was expressed as µg/ml egg-white lysozyme, but since human lysozyme has about 3.5 times the activity of egg-white lysozyme (Jollès and Jollès, 1967) the figures do not represent true concentrations.

Normal Values

In an earlier study (Harrison *et al*, 1968) from 24 estimations each the normal range for serum lysozyme was considered to be 5.1–14 µg/ml, and that for urine was 0.07–1.1 µg/ml. Using the present slightly modified method and random 'normal' specimens arriving in the hospital biochemistry laboratory, the normal range was $9.4 \pm \text{SD } 2.7$ µg/ml for serum (55 estimations), and $0.7 \pm \text{SD } 0.6$ µg/ml for urine (82 estimations). We have therefore defined 'lysozymuria' as a urine lysozyme concentration greater than 1.9 µg/ml.

Evidence that Lysozymuria Was Not Caused by Serum Lysozyme Levels above the Normal Renal Threshold

The normal renal threshold for lysozyme is not easy to determine. There have been no studies based on lysozyme infusions to raise the serum concentration in human subjects. Experiments with egg-white lysozyme infused into dogs (Harrison and Barnes, 1970) have suggested a threshold corresponding to 32–56 µg/ml for human lysozyme, allowing for the difference in activity of the two lysozymes, but canine

and human thresholds may not be the same. Since very high levels are found in leukaemia it should be possible to deduce the threshold using data from leukaemic patients. In this way there have been various estimates: 45 µg/ml (Hayslett *et al*, 1968), more than 50 µg/ml (Perillie, Kaplan, Lefkowitz, Rogaway, and Finch, 1968), and variable but not less than 42 µg/ml (Wiernik and Serpick, 1969).

Our own data from patients with leukaemia do not give conclusive evidence: they are shown in figure 1. Abnormally high urinary lysozymes occurred with serum levels as low as 18 µg/ml; they were presumably due to renal tubular defects since normal urinary excretion occurred with serum concentrations up to 52 µg/ml. Pruzanski and Platts (1970) have similarly found evidence of tubular dysfunction in leukaemia.

Fig 1 Lysozyme concentrations in paired serum and urine specimens obtained simultaneously from patients with leukaemia. The data do not give conclusive information above the renal lysozyme threshold, but are compatible with evidence from the literature suggesting that it is at least 45 µg/ml.

Taking all the evidence together, it seems reasonable to conclude that lysozymuria may be considered evidence of abnormal tubular function if the serum lysozyme is below 45 µg/ml.

Results

INCIDENCE OF LYSOZYMURIA IN CONDITIONS WHICH MIGHT PREDISPOSE TO TUBULAR DYSFUNCTION

In this group sera and urines were collected in two principal conditions: postoperatively and after acute myocardial infarction. Patients with pneumonia, hypokalaemia, salicylate poisoning, paracetamol poisoning, and jaundice were also included.

Operative surgery

Thirty six patients were studied: 25 of them underwent elective operations and 11 were operated on as

	Number of Cases with Urine Lysozyme <2.0 µg/ml		Number of Cases with Urine Lysozyme >1.9 µg/ml		Maximum Urine Lysozyme (µg/ml)
	Serum Urea <40 mg/100 ml	Serum Urea >39 mg/100 ml	Serum Urea <40 mg/100 ml	Serum Urea >39 mg/100 ml	
<i>Elective Surgery</i>					
Cholecystectomy	2	2	2	1 ¹	7.4, 8.4, 7.1 ¹
Abdominal aneurysm	1			1 ¹	12.0 ¹
Other abdominal operations	6	3		1 ¹	4.2 ²
Non-abdominal operations	3	3			
<i>Emergency Surgery</i>					
Appendicitis	4		1 ⁴	2 ³	9.6, 32.0, 12.5 ³
Perforated ulcer	1		1		2.0
Normal appendix	1				
Pancreatitis	1				

Table I Incidence of lysozymuria following 36 surgical operations

¹These patients had each an episode of postoperative 'collapse' with transient hypotension.²This patient had a severe wound infection.³One patient became seriously electrolyte-depleted.⁴Serum urea not determined in this case.

emergencies. Specimens were collected preoperatively and for one to four days postoperatively. None of the serum lysozyme levels were greatly raised (2.4–21.6 µg/ml), and the preoperative urinary lysozymes were all normal except for one patient with a perforated ulcer. The results of the postoperative urinary lysozyme determinations are shown in table I. Concentrations above 1.9 µg/ml were found in two patients who had postoperative 'collapse', in one patient with severe wound infection, in one patient who became severely electrolyte-depleted, and also in five patients who had apparently uneventful operations—two routine cholecystectomies, two appendectomies, and one perforated ulcer repair. The urine lysozyme concentration reached a very variable maximum, and by the end of the period of observation had returned to normal in only three of the nine cases (one cholecystectomy, aneurysm, perforated ulcer). Twenty-seven other patients, some of whom underwent the same operations, did not have lysozymuria. The daily urinary output in every case was greater than 1 litre, and lysozymuria was unrelated to serum urea or creatinine levels. Tests for proteinuria were not carried out.

Myocardial infarction

Data were obtained from 19 patients for two to eight

days following infarction. Serum lysozyme levels were all normal or only slightly raised (8.8–16.2 µg/ml), except for one patient who had a serum lysozyme level of 46.2 µg/ml, and his urinary lysozyme was normal. (His data were incomplete and are not included in the following analysis.) Table II summarizes the information: the highest urinary lysozyme level was 5.0 µg/ml. Raised levels following abolition of ventricular fibrillation by DC shock did not last longer than 24 hours; none of the three patients became oliguric, and there was a further similar patient who did not have lysozymuria. Five patients had severe heart failure, of whom three had lysozymuria, which in one case was transient; in the other two it was intermittent, and these were the only patients whose 24-hour urine volumes fell for a time below 400 ml. Lysozymuria was always accompanied by proteinuria, but proteinuria also occurred intermittently without any increase in the urinary lysozyme, even in apparently uncomplicated cases.

Severe pneumonia

Serum and urinary lysozyme levels remained normal in three cases of mild chest infection, and in three other cases of mild febrile illness. Two cases of lobar pneumonia, however, had brief episodes of lysozymuria, and the relevant data are summarized in table

	Number of Cases with Urine Lysozyme <2.0 µg/ml	Urine Lysozyme >1.9 µg/ml		
		Number of Cases	Maximum Level	Duration
Uncomplicated infarct	9	0		
Ventricular fibrillation abolished by DC shock	1	3	2.3, 3.7, 4.4	24 hours
Infarction complicated by severe heart failure	2	3	2.0 2.2, 5.0	48 hours intermittent

Table II Incidence of lysozymuria following 18 episodes of myocardial infarction

	Serum Lysozyme ($\mu\text{g/ml}$)	Serum Urea ($\text{mg}/100\text{ml}$)	Urine Lysozyme ($\mu\text{g/ml}$)	Urine Protein ($\text{mg}/100\text{ml}$)	Temperature ($^{\circ}\text{C}$)
Case 1 Day 1			14.2	100	40.0
Day 2	11.2	34	1.8	<10	36.8
Day 3	12.8		0.6	<10	36.8
Day 4	14.4		0.4	<10	36.8
Case 2 Day 1			10.0	40	38.6
Day 2	11.2	82	14.2	30	38.0
Day 3	19.2		5.2	<10	38.4
Day 4	17.6		1.4	<10	37.5

Table III Data from two patients with lobar pneumonia also with transient lysozymuria but not oliguric

III. Both these patients were promptly treated with antibiotics and made uncomplicated recoveries; both had serum creatinines below $1.1 \text{ mg}/100 \text{ ml}$ and urine urea concentrations above $2 \text{ g}/100 \text{ ml}$, and neither of them was observed to be oliguric.

Hypokalaemia

An opportunity was taken to obtain data from a patient with hypokalaemia (serum K^+ 1.7 m-equiv/l) of unknown cause, while he was being repleted with potassium. His serum urea was normal and so was his serum lysozyme. Figure 2 shows the data: there was lysozymuria which disappeared quickly as the serum potassium level rose, and the patient's proteinuria diminished at the same time.

Fig 2 Data from a patient with hypokalaemia of unknown cause while he was being repleted with potassium.

Other observations

Urinary lysozyme levels remained normal in two cases of salicylate poisoning, three cases of paracetamol poisoning, and one case of obstructive jaundice (serum bilirubin $23 \text{ mg}/100 \text{ ml}$).

THE INCIDENCE OF LYSOZYMURIA IN 'EXTRARENAL URAEMIA'

Sera and urines were collected in two principal conditions in which the serum urea was raised: acute illnesses characterized by dehydration and electrolyte depletion, and severe heart failure.

Lysozymuria accompanying uraemia and electrolyte depletion

Table IV outlines the data obtained from seven patients, and the sequence of events in two of them is given in more detail in figure 3. Surgical operations were performed before the period of observation in two cases. Each patient had a raised urinary lysozyme level which was followed until it returned to normal or until the patient left hospital. Its relationship to the serum urea level, and other details of the clinical picture, varied from case to case. Again, lysozymuria and proteinuria accompanied one another; in six cases all the serum lysozyme levels were below the normal renal threshold, but the threshold was exceeded by the only serum lysozyme estimation in the case of patient EC. The urinary volume remained normal in six cases, but patient PG was anuric for about 48 hours. In three cases the lysozymuria 'peak' occurred four or five days after the highest serum urea, and after the patients had been repleted with fluid. The urinary lysozyme returned to normal in

Fig 3 Data from two patients with transient uraemia complicating dehydration and electrolyte depletion. Information on TM's urinary output is deficient, but he was not observed to be oliguric. GB's urinary lysozyme did not return to normal.

Patient Diagnosis		Serum Urea			Urine Lysozyme		
		Highest Level (mg/100 ml)	Final Level (mg/100 ml)	Duration of Rise in Days	Highest Level (μg/ml)	Final Level (μg/ml)	Duration of Rise in Days
TM	Postoperative biliary fistula	235	22	13	11.3	0.8	9
ES	Intestinal obstruction (postoperative)	174	24	26	9.2	0.9	<23
GB	Jaundice and vomiting	198	37	6	27.0	4.4	9
EC	Oesophageal obstruction and vomiting	292	26	4	23.2	0.5	<23
PG	Severe gastroenteritis	274	26	17	12.7	0.5	<17
IP	Gastroenteritis; diabetes	248	60	26	13.6	12.0	— ¹
WM	Diabetic ketosis	82	46	2	23.0	0.7	3

Table IV Data from seven patients with acute illnesses characterized by dehydration

¹This patient's urinary lysozyme slowly rose for three weeks despite apparent recovery. Later she died, and postmortem examination revealed a renal abscess.

five cases but in two it did not: in patient IP it continued slowly to rise despite apparent improvement and the disappearance of proteinuria: a low-grade fever continued, and the patient later died from peritonitis caused by a ruptured renal abscess.

Lysozymuria accompanying uraemia and heart failure

Data were collected from seven patients with severe heart failure whose maximum serum urea levels varied between 75 and 212 mg/100 ml. Four of them had intermittent slight proteinuria (not more than 15 mg/100 ml) but none had lysozymuria. There were also the five patients with heart failure complicating myocardial infarction shown in table II, of whom three had slight, transient, or intermittent lysozymuria; their maximum serum urea levels were between 75 and 127 mg/100 ml.

LYSOZYMURIA IN ACUTE RENAL DISEASE

During the period of this study a miscellaneous group of patients with acute, apparently primary, renal disease presented themselves, and the data from some of them are summarized in table V. Each nephrotic patient presented with massive oedema and was given diuretic treatment: each one had lysozymuria, which diminished with the diuresis. The urinary

lysozyme was normal in two cases of acute glomerulonephritis, but lysozymuria occurred transiently in a third case. Only one or two specimens were obtained from each of the patients with pyelonephritis so the duration of their lysozymuria is uncertain, but it was probably brief. The acute exacerbation of chronic renal disease was induced by urinary tract infection in a patient with phenacetin nephropathy: the urine lysozyme peak was very high indeed, and occurred 13 days after the highest serum urea level. Serum lysozyme levels were high in several of these patients, but remained below the presumed normal threshold.

Discussion

The results show that transient lysozymuria occurs frequently among patients with acute illnesses, many of whom show no other evidence of renal disease. If one accepts the arguments outlined above, that lysozymuria is nearly always a sign of impaired proximal tubular function, then acute reversible tubular lesions must be common. Most of the conditions in which there was pronounced lysozymuria (more than 5 μg/ml) were those which are sometimes associated with anuric tubular necrosis—surgical operations complicated by hypotension or severe

		Serum Urea (mg/100 ml)		Urine Lysozyme (μg/ml)		Duration of Peak (days)	Highest Serum Lysozyme (μg/ml)
		Highest	Final	Highest	Final		
MM	Nephrotic syndrome with oedema	85	36	4.7	0.6	15	—
BK		23	12	11.7	0.8	22	—
RP	Acute glomerulonephritis	120	—	0.6	—	—	24.0
DH		—	—	4.4	1.0	<3	8.7
BM		330	140	1.8	—	—	34.4
BA	Acute pyelonephritis	10	—	6.9	—	—	5.6
JS		18	—	10.5	1.1	<5	16.0
YR		18	—	8.0	0.7	?	5.6
SF		14	—	9.8	—	—	24.0
EM	Acute on chronic renal failure	420	48	87.0	14.3	61	43.2

Table V Data from a miscellaneous small group of patients with acute renal disease

Relationship of Urine Lysozyme Peak to Serum Urea Peak	Urine Protein		Serum Lysozyme Highest Level ($\mu\text{g/ml}$)	Observed Urine Volume (l/24 hr)
	Highest Level (mg/100 ml)	Final Level (mg/100 ml)		
4 days later	35	0	18.0	1.22-2.20
4 days later	35	0	22.4	1.32-2.35
1 day later	100	30	18.4	1.50-1.82
5 days later	60	0	71.2	Not recorded
Uncertain	150	0	29.6	0.4-4.0
— ¹	10	0	18.4	1.03-2.90
Same day	80	0	20.8	0.64-1.73

Table IV Data from seven patients with acute illnesses characterized by dehydration—continued

infection, electrolyte depletion, the nephrotic syndrome, acute pyelonephritis. Therefore in these patients the lysozymuria may well have represented tubular necrosis which was irregularly distributed or incomplete. On the other hand not all the alterations of function might have been due to abnormalities which if severe would inevitably lead to cell death; this may apply in particular to hypokalaemia, which is not generally associated with anuria. Only one of the patients described had a renal biopsy; she had the nephrotic syndrome, and there were quite large areas of recovering tubular damage where the tubules were lined by a low simple type of epithelium. Hayslett *et al* (1968) have described lysozymuria coupled with similar biopsy appearances in the nephrotic syndrome and acute glomerulonephritis.

If it is accepted that lysozymuria represents tubular proteinuria, then proteinuria (detected for example by salicysulphonic acid) should have accompanied lysozymuria in every case. In fact on a few occasions lysozymuria apparently occurred alone, and in one such instance the patient ultimately proved to have a renal abscess. There was no record of her urinary white count cell count, but the case raises the question whether the presence of pus in large amounts, either in the kidney or in the urine, might cause secretion of lysozyme without other proteins, due to the breakdown of leucocytes; further work is necessary to decide if this can happen. With the exception of such patients we would anticipate results like ours if other low molecular weight urine proteins were studied in a similar group of patients with acute disease, using for example an immunochemical method as described by Peterson, Evrin, and Berggård (1969).

Like other proteins, reabsorbed lysozyme is catabolized in the renal tubules, so that lysozyme which passes into the glomerular filtrate is 'lost' to the circulation whether it is excreted in the urine or not (Harrison and Barnes, 1970). The serum lysozyme concentration accordingly depends on the glomerular filtration rate but not on tubular function; in consequence, it tends to rise with advancing azotaemia. Some patients with renal failure might

therefore be expected to have lysozymuria because of 'overflow' without necessarily a disproportionate loss of tubular function. In fact nearly all the patients described above had serum lysozyme levels below 45 $\mu\text{g/ml}$, and there was no correlation between urinary lysozyme and the concentration of creatinine, urea, or lysozyme in serum. Combining the results from these patients with a larger, also miscellaneous group, some of whom had advanced chronic renal failure, among 263 estimations of serum lysozyme there were only seven with levels above 45 $\mu\text{g/ml}$ and only four with levels above 50 $\mu\text{g/ml}$: these four included three specimens from three patients with dehydration (one of whom is included in table IV), and a specimen from a patient with hyperosmolar diabetic coma. High serum lysozymes also occur in the early stages of acute tubular necrosis (Hayslett *et al*, 1968; Harrison *et al*, 1972). Under these circumstances, lysozymuria may not necessarily be due to impaired tubular reabsorption. Therefore when evidence of tubular damage is sought by estimating the urinary lysozyme, strictly the serum lysozyme should also be known, although in most clinical circumstances the chances are very high that it will be below the normal threshold.

A particular motive for undertaking the study was to find whether lysozymuria is a useful sign of impending oliguric tubular necrosis or has any other diagnostic value in acute illness, for example, in distinguishing tubular necrosis from acute oliguric glomerulonephritis. In these respects the results have been disappointing: although raised urinary lysozymes were discovered frequently, hardly any of the patients were oliguric at any stage, and since lysozymuria occurred in acute nephritis it cannot reliably distinguish between primarily glomerular and primarily tubular disease. On the other hand, a normal urinary lysozyme should at least make tubular necrosis an unlikely diagnosis, although this observation is not likely to be very helpful in clinical practice. Hayslett *et al* (1968) and Wauters and Favre (1970) have found that dehydration is unaccompanied by lysozymuria, but our electrolyte-

depleted, dehydrated patients had pronounced lysozymuria and again the distinction does not appear clinically useful. Similarly among patients with renal transplants, lysozymuria cannot be regarded as a specific sign of transplant rejection (Harrison *et al*, 1972). We did not think it would help to measure the excretion rate or the clearance of lysozyme instead of the urine concentration, mainly because there is so little lysozyme in normal urine: the observed increases in lysozyme excretion were mostly so great that the extra information given by these more complicated estimates would have been hardly significant.

The results are perhaps of more interest in helping to understand renal pathophysiology in acute disease, and particularly in showing the vulnerability of renal tubular cells to impairment of their function. Whatever the exact nature of the tubular abnormalities, the causes must have been various. Hypokalaemia is a well known cause of tubular lesions and presumably interferes with cell enzymes and transport mechanisms. The way in which dehydration and electrolyte depletion cause damage is less certain: patients in this group had some of the most pronounced lysozymuric episodes, but apart from the patient with diabetic ketosis their serum potassium levels were normal. Some of them excreted the largest amounts of lysozyme during recovery, which is consistent with the hypothesis that they had patchy tubular necrosis with urea retention and localized failure of urine secretion: if this were so, the damaged tubules would have allowed urine to flow again as recovery began, but at first with deficient protein reabsorption. In acute pyelonephritis, oedema, and inflammation of the kidney are likely to have been responsible for tubular damage. The reversible lysozymuria of the nephrotic patients might have been related either to renal oedema or to some kind of intracellular electrolyte imbalance. The cause of the lysozymuria accompanying severe pneumonia and wound infection is also uncertain; perhaps circulating toxins were involved. These cases of infection incidentally suggest that the well recognized proteinuria of acute fevers is due to transient tubular damage. Two surgical patients had lysozymuria without any apparent complication to their operations, and there was no reason to suspect the anaesthetic: again, the cause is obscure.

All these patients contrast with those who had heart disease, most of whom had normal urinary lysozymes even though many of them were azotaemic. Cardiac arrest (which lasted perhaps 10 minutes in each case before resuscitation) might be expected to cause anoxic tubular damage, but lysozymuria was either absent or slight and of brief duration; it was

also an exceptional finding in even the severest cases of heart failure, and these patients' azotaemia could therefore perhaps truly be regarded as 'extrarenal', caused by a low cardiac output. It was also of interest to discover that poisoning with aspirin and paracetamol, and severe but otherwise uncomplicated jaundice, did not appear to cause tubular damage.

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